Manipulation of Endophytic Bacteria for Symbiotic Control of *Xylella fastidiosa*, Causal Agent of Citrus Variegated Chlorosis

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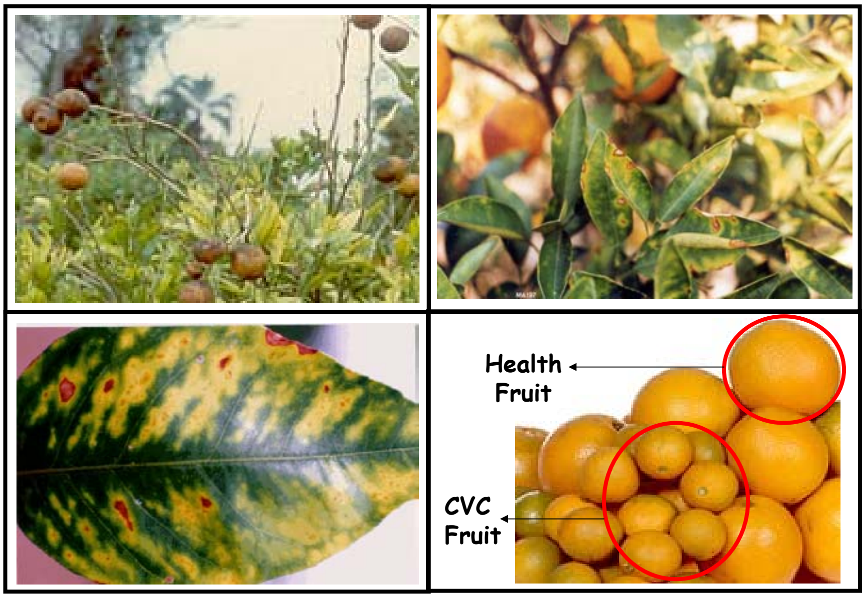
Citrus Variegated Chlorosis (CVC)

Bacterium
Xylella fastidiosa

Colonize the xylem vessels, forms biofilms → **Yield reduction and plant death**

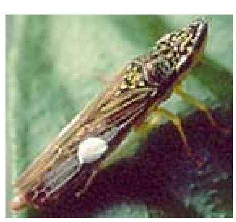
Brazil loses about US$ 200 million per year to CVC !!!

Disease Symptoms



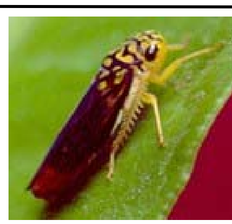
Insect Vectors of *X. fastidiosa* (CVC)

Sharpshooter leafhoppers (Homoptera, Cicadellidae, subfamily Cicadellinae)

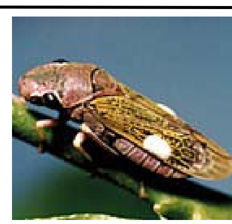


Acrogonia citrina

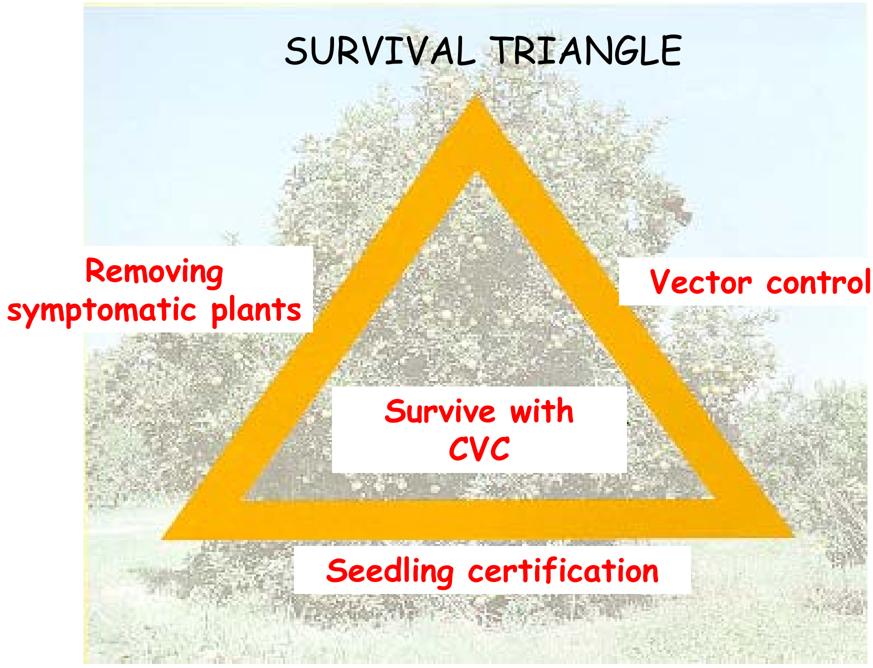
Bucephalogonia xanthophis



Dilobopterus costalimai



Oncometopia facialis

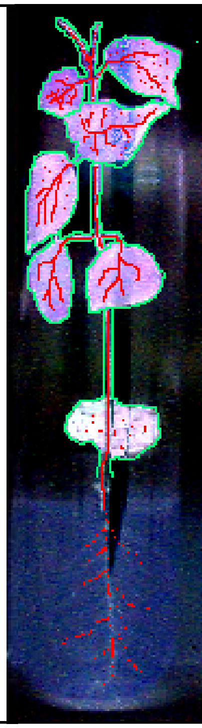


Endophytic Microorganisms

Definition

Hallmann et al. (1997): microorganisms that can be isolated from surface desinfected plant tissues and do not induce any visible harm to them

Azevedo et al. (2000): microorganisms inhabit inside plant tissues without neither causing disease to the host nor producing visible external structures.



Host plant

Microorganisms stock center

Epiphytic microorganisms

Endophytic microorganisms

Endophytes colonize ecological niches similar to those colonized by phytopathogens

Application of endophytic microorganisms

Biological control of plant pathogens

- Antimicrobial compounds
- Inducing systemic resistance

Plant-Microbe Interactions, 2008: ISBN: 978-81-7895-314-4
Editors: E. Ait Barka and C. Clément

5 Interaction between endophytes and plant host: Biotechnological aspects

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Fernando Dini Andreote[2] and João Lúcio Azevedo[1,2]
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How endophytes could be used as biological control agents of CVC

- Select endophytic bacteria to be used in biological control of CVC
- Develop a model system to study the interaction between endophytic bacteria and *X. fastidiosa*
- CVC control depends on the localization of the endophytic bacteria close to *X. fastidiosa* niche

- **Previous Endophytes Study in Citrus Leaves** (Araújo et al. 2001)
  - *Pantoea agglomerans*
  - *Bacillus pumilus*

- **Endophytes in Citrus Stems** (Araújo et al. 2002)
  - *Methylobacterium spp.*
  - *Curtobacterium flaccumfaciens*

Interaction between endophytic bacteria from citrus plants and the phytopathogenic bacteria *Xylella fastidiosa*, causal agent of citrus-variegated chlorosis

P.T. Lacava[1], W.L. Araújo[1], J. Marcon[1], W. Maccheroni Jr[1] and J.L. Azevedo[1,2]
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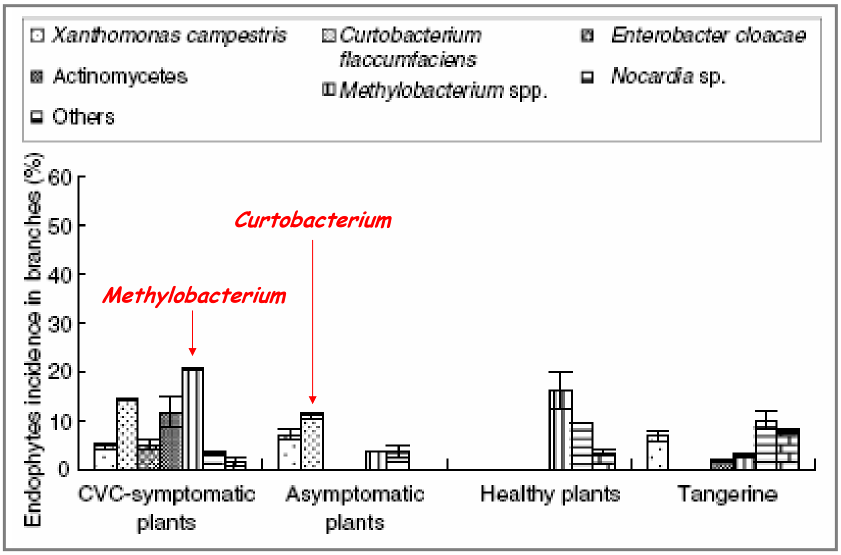
ABSTRACT

P.T. LACAVA, W.L. ARAÚJO, J. MARCON, W. MACCHERONI JR AND J.L. AZEVEDO. 2004.
Aims: To isolate endophytic bacteria and *Xylella fastidiosa* and also to evaluate whether the bacterial endophyte community contributes to citrus-variegated chlorosis (CVC) status in sweet orange (*Citrus sinensis* [L.] Osbeck cv. Pera).
Methods and Results: The presence of *Xylella fastidiosa* and the population diversity of culturable endophytic bacteria in the leaves and branches of healthy, CVC-asymptomatic and CVC-symptomatic sweet orange plants and in tangerine (*Citrus reticulata* cv. Blanco) plants were assessed, and the *in vitro* interaction between endophytic bacteria and *X. fastidiosa* was investigated. There were significant differences in endophyte incidence between leaves and branches, and among healthy, CVC-asymptomatic and CVC-symptomatic plants. Bacteria identified as belonging to the genus *Methylobacterium* were isolated only from branches, mainly from those sampled from healthy and diseased plants, from which were also isolated *X. fastidiosa*.
Conclusions: The *in vitro* interaction experiments indicated that the growth of *X. fastidiosa* was stimulated by endophytic *Methylobacterium extorquens* and inhibited by endophytic *Curtobacterium flaccumfaciens*.
Significance and Impact of the Study: This work provides the first evidence of an interaction between citrus endophytic bacteria and *X. fastidiosa* and suggests a promising approach that can be used to better understand CVC disease.

Letters in Applied Microbiology 2004, **39,** 55–59

Isolation of culturable endophytic bacteria



Incidence of the main endophytes isolated from branches of sweet orange and tangerine plants

CONCLUSION (Lacava et al , 2004):

- *X. fastidiosa* may colonize CVC-asymptomatic plants without inducing symptoms of CVC;

- *Methylobacterium* spp. were only isolated from branches and mainly from CVC-symptomatic;

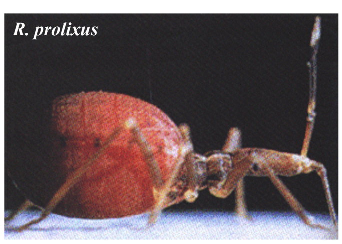
- *C. flaccumfaciens* inhibited *in vitro X. fastidiosa*. The CVC symptoms could be reduced by a high population of this endophytic bacterium.

Symbiotic Control

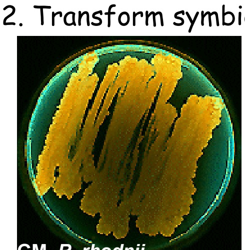
- Relies on the use of symbionts (transformed or not) to reduce vector competence;
- Pioneered by Frank Richards at Yale University;
- Trial to suppress Chagas disease through paratransgenesis;
- Paratransgenesis:
  * Genetic modification of symbiotic bacteria to make the host less competent in transmitting a pathogen.

Paratransgenesis: The Chagas Disease Case

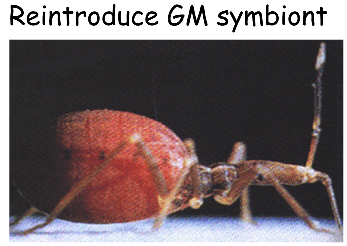
1- Identify and isolate symbiont



2. Transform symbiont.



3. Reintroduce GM symbiont



Source: slide from J.L. Ramirez

Symbiotic Control

Requirements for a successful paratransgenic approach:

1- Symbiont selection
   * Commensal or mutualistic relationship.
   * Contact symbiont-pathogen;

2- Symbiont culturable and amenable for GM;

3- Uncompromised GM symbiont fitness;

4- Methods for reintroduction and spread of the GM symbiont.

Beard et al, 2001

CURRENT MICROBIOLOGY Vol. 48 (2004), pp. 327–331
DOI: 10.1007/s00284-003-4178-2

Current Microbiology
An International Journal

Delivery of a Genetically Marked *Alcaligenes* sp. to the Glassy-Winged Sharpshooter for Use in a Paratransgenic Control Strategy

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Received: 23 June 2003 / Accepted: 15 August 2003

CURRENT MICROBIOLOGY Vol. 50 (2005), pp. 1–7
DOI: 10.1007/s00284-004-4390-8

Current Microbiology
An International Journal

Establishment of a Genetically Marked Insect-Derived Symbiont in Multiple Host Plants

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Strategies of control of *CVC* using endophytes

Classic Biocontrol

↓

Curtobacterium

(endophytic bacteria from citrus)

↓

***Curtobacterium* inhibit *in vitro* Xf (Lacava et al, 2004)**

↓

Tested in model plant in greenhouse (Lacava et al, 2007)

↓

Next step: test in the field in citrus plants

Symbiotic Control

↓

Methylobacterium

(endophytic bacteria from citrus)

↓

***Methylobacterium* can colonize plant tissue and sharpshooter (Andreote et al, 2006 and Gai, 2006)**

↓

Transformation of *Methylobacterium* (GFP)

↓

Next step: production of anti-*Xylella* effectors

The Journal of Microbiology, October 2007, p. 388-393 Vol. 45, No. 5

The Endophyte *Curtobacterium flaccumfaciens* Reduces Symptoms Caused by *Xylella fastidiosa* in *Catharanthus roseus*

Paulo Teixeira Lacava[1*], Wenbin Li[2], Welington Luiz Araújo[1], João Lúcio Azevedo[1], and John Stephen Hartung[3]

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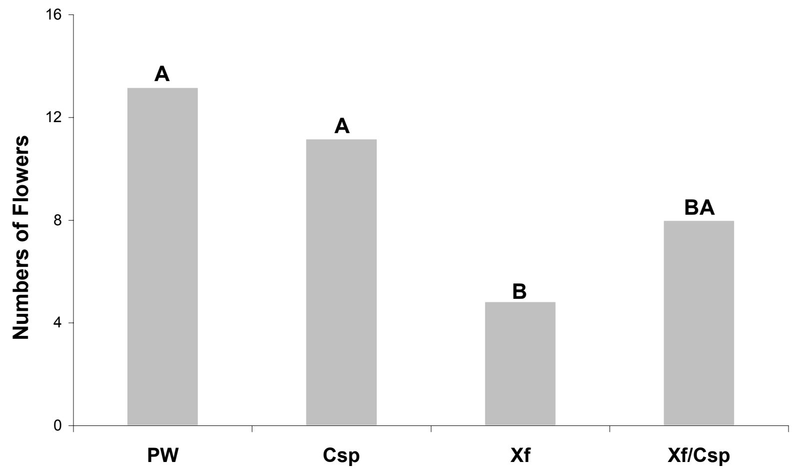
Poster # 56

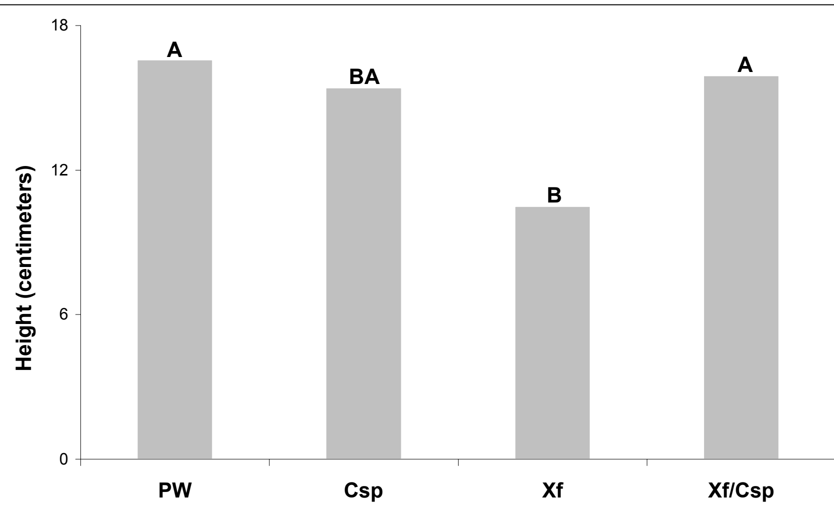
RESULTS:



PW: media; Csp: *C. flaccunfaciens*; Xf: *X. fastidiosa*; Xf/Csp: *X. fastidiosa* and *C. flaccunfaciens*

Interaction between *C. flaccunfaciens* (endophytic bacteria) and *X. fastidiosa* inside of host plant (*C. roseus*). Different letters on bars show statistic difference by Tukey's test at 5% of significance

RESULTS:



PW: media; Csp: *C. flaccunfaciens*; Xf: *X. fastidiosa*; Xf/Csp: *X. fastidiosa* and *C. flaccunfaciens*

Interaction between *C. flaccunfaciens* (endophytic bacteria) and *X. fastidiosa* inside of host plant (*C. roseus*). Different letters on bars show statistic difference by Tukey's test at 5% of significance

RESULTS:

Figure 6. Disease symptoms of induced in *Catharanthus roseus* plants 2 months after inoculation with *X. fastidiosa* (right). A symptom free plant doubly-inoculated *X. fastidiosa* and *C. flaccumfaciens* is also shown (left).

RESULTS:

Figure 7. Leaf stunting and chlorosis induced in *Catharanthus roseus* leaves 2 months after inoculation with *Xylella fastidiosa* (left). Symptom free leaves from a plant doubly-inoculated with *X. fastidiosa* and *C. flaccumfaciens* (right).

CONCLUSIONS:

• The results reinforce the idea suggested by Araújo et al. 2002 and Lacava et al. 2004 that this endophytic bacterium, that colonizes a niche similar to that of *X. fastidiosa*, could contribute to the reduction of the symptoms of CVC in the field.

Manipulation of Endophytic Bacteria for Symbiotic Control of Citrus Variegated Chlorosis

Cláudia Santos Gai[a], Paulo Teixeira Lacava[a*], Fernando Dini Andreote[a], Maria Carolina Quecine[a], Marie-Christine Auriac[b], João Roberto Spotti Lopes[a], Welington Luiz Araújo[a,c], João Lúcio Azevedo[a,c]

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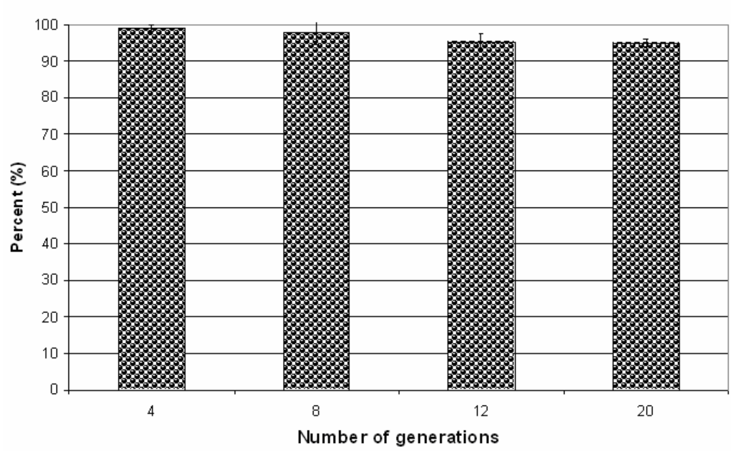
Manuscript Draft

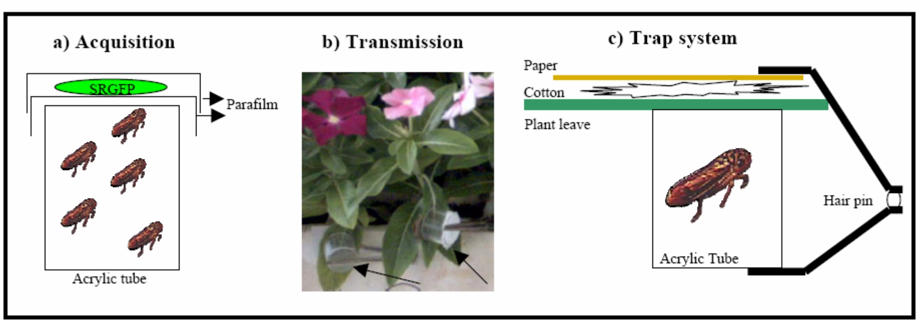
Poster # 58

RESULTS:



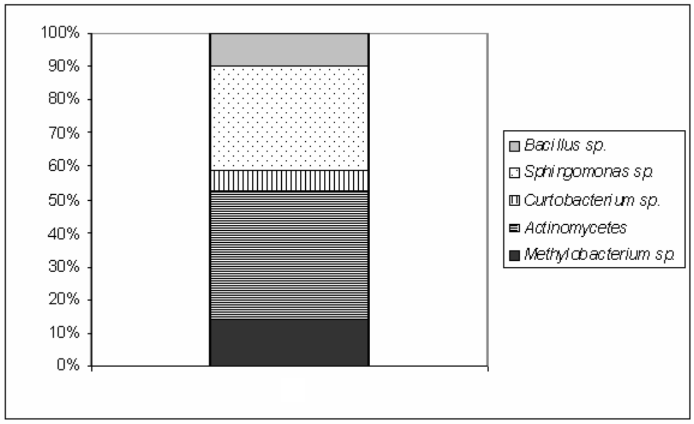
Plasmidial stability of pCM88 on *Methylobacterium mesophilicum*. The percent was obtained colleting randomly samples after 24, 48, 72 and 120 hours of culture cells of SRGFP strain growing without antibiotic tetracycline.

RESULTS:



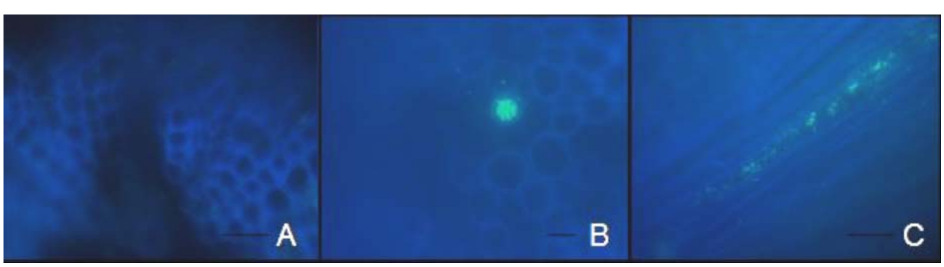
Transmission assay. a) Membrane system used for insect feeding, adapted from Purcell and Finlay, 1979. b) Plants with traps containing insects that fed on SRGFP. Arrows indicating traps positioned on plant leaves. c) Detail of trap system.

RESULTS:



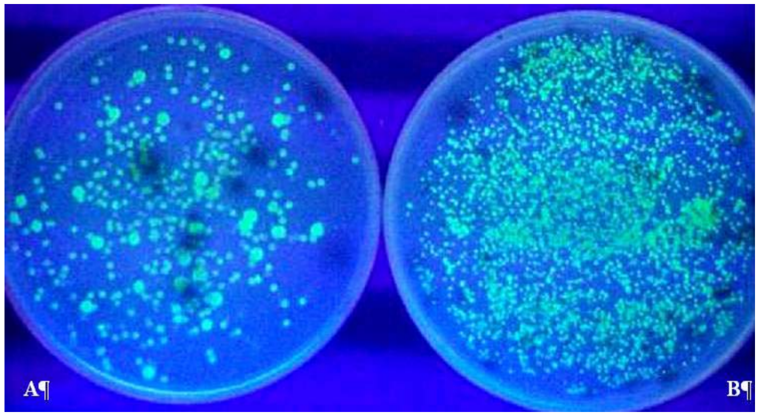
Most frequently group of bacteria isolated from *Bucephalogonia xanthophis*.

RESULTS:



Fluorescent microscopy evidencing the ecological niche occupied by endophyte *Methylobacterium mesophilicum* in *Catharanthus roseus* plants 45 days after inoculation. A) Xylem vessels of a control plant, Scale bar = 10μm; B) Colonized xylem vessel, Scale bar = 5μm; C) Xylem vessels longitudinal cut, Scale bar = 10μm.

RESULTS:



Insects were submitted to isolation form confirmation of the presence of SRGFP colonizing their heads. A) Plate with fluorescent bacteria isolated from insect head after feeding on membrane system. B) Isolation after plant feeding during 24 hours.

Suggestions and Conclusions:

• This is the first report of genetic manipulation of endophytic *M. mesophilicum* isolated from citrus plants;

• *In vitro* the pCM88 plasmid was stably maintained in SRGFP strain for at least 20 generations of cells growing in medium without antibiotic;

• SRGFP strain was stable after the bacteria colonized of plant and insect tissues, therefore representing an important tool to assess both the establishment of the inoculated strain in host plant and the transmission by vector;

• As showed in this study, the transgenic endophytic *M. mesophilicum* has most of the prerequisites for a successful strategy using paratransgenesis (symbiotic control);

• *M. mesophilicum* that colonize citrus plants (Araújo et al. 2002; Lacava et al. 2004) and *B. xanthophis* is amenable to isolation, culture and transformation with foreign genes.

Entomological Research 37 (2007) 261–266

RESEARCH PAPER

Analysis of the bacterial community in glassy-winged sharpshooter heads

Paulo Teixeira LACAVA[1], Jennifer PARKER[2], Fernando Dini ANDREOTE[1], Francisco DINI-ANDREOTE[1], José Luiz. RAMIREZ[2] and Thomas A. MILLER[2]

1 Department of Genetics, Escola Superior de Agricultura "Luiz de Queiroz", University of São Paulo, Piracicaba, São Paulo, Brazil
2 Department of Entomology, University of California Riverside, Riverside, California, USA

• Candidate endophytes for use in SC must occupy the xylem of host plants and attach to the pre-cibarium and cibarium of sharpshooter insects in order to have access to the pathogen;

• The use of isolation and DGGE techniques revealed several genera of bacteria as colonizers of GWSS heads. As identified by 16S sequencing, these included, *Methylobacterium extorquens* and *Curtobacterium flaccumfaciens*;

• Species such as *Curtobacterium flaccumfaciens* and *Methylobacterium* spp., found in part of the bacterial community in GWSS, could be investigated as potential candidates for use in an SC-based strategy to control the spread of PD.

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• Prof. Dr. Thomas A. Miller, University of California Riverside, Riverside, CA, USA

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